UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/566,898	10/26/2006	Ellen Jessouroun	NIH275.001NP2	9576

3000 7590 07/27/2010

CAESAR, RIVISE, BERNSTEIN,
COHEN & POKOTILOW, LTD.
11TH FLOOR, SEVEN PENN CENTER
1635 MARKET STREET
PHILADELPHIA, PA 19103-2212

EXAMINER	
ARCHIE, NINA	

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
07/27/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patents@crbcp.com

Office Action Summary	Application No.	Applicant(s)	
	10/566,898	JESSOUROUN ET AL.	
	Examiner	Art Unit	
	Nina A. Archie	1645	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *07 April 2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-20* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-20* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. This Office Action is responsive to Applicant's amendment and response filed 4-7-10. Claims 1, 6-8, and 15-16 have been amended. Claims 1-20 are pending and under examination.

Objections/Rejections Withdrawn

2. In view of the Applicant's amendments and remarks the following objections/rejections are withdrawn.

a) Rejection to claims 1-20 under 35 U.S.C. 112, second paragraph, for the recitation "of from about" as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is withdrawn in light of applicant's amendment thereto.

Claim Rejections Maintained

35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

3. The rejections of claims 9 and 17 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention are maintained for the reasons set forth in the previous office action.

Applicants arguments filed in response to the 35 U.S.C. 112, second paragraph, April 7, 2010 is carefully considered, but not found to be persuasive for the reasons below.

Applicant argues:

A) With regard to the term "substantially", Applicants acknowledge that the specification does not provide an explicit definition. However, a person of ordinary skill in the art (a "POSA") would have understood from the original disclosure that the term "substantially" simply recognizes the practical limits of the diafiltration step recited in the claims. The POSA would have accordingly interpreted the expression "substantially all unreacted compounds and

unconjugated polysaccharides are removed" as covering the removal of all such moieties, or at least as much as can be removed within the practical limitations of diafiltration.

Examiners Response to Applicants Arguments:

With regard to Point (A), Applicants are using "substantially" which is a relative term to define a relative term. Applicants have not clarified a significant degree in the recitation "substantially all unreacted compounds and unconjugated polysaccharides are removed" represents. Therefore, Examiner does not know what constitutes as "substantially all unreacted compounds and unconjugated polysaccharides are removed", thus Examiner would not know what substantially means. Moreover a person with ordinary skill in the art would not know what was meant by "substantially all unreacted compounds and unconjugated polysaccharides are removed". Therefore the rejection is maintained.

As outlined previously, as to dependent claims 9 and 17, the claims are rendered vague and indefinite by the use of the term "substantially". The term "substantially" is a relative term that is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

4. The rejection of claims 1-4 and 8-14 under 35 U.S.C. 103(a) as being unpatentable over (Schwartz US Patent No. 6,800,728 Date October 5, 2004 US Filing Date March 22, 2001),

(Ryall et al US Patent No. 5,965,714 Date October 12, 1999), (Kuriyama et al US Patent No. 4,963,232 Date October 16, 1990), as evidenced by (Behr et al 2003 Tetrahedron 59 pgs. 543-553) is maintained for the reasons set forth in the previous office action.

Applicants arguments filed in response to the 35 U.S.C. 103(a), April 7, 2010 is carefully considered, but not found to be persuasive for the reasons below.

Applicant argues:

A) Schwartz does not disclose all claimed features. Although Schwartz describes the conjugation between oxidized polysaccharide and NaIO4 and modified protein with hydrazine (see example 29), the reaction does not occur by the mechanism of reductive amination, i.e., the connection between the protein modified by hydrazine and polysaccharide activated by NaIO4 occurs in the absence of reducing agent. Thus, Schwartz fails to disclose or suggest the claimed feature of reacting the aldehyde-activate polysaccharide with the hydrazine-activated protein at a pH of from 5 to 7 in the presence of the reducing agent sodium cyanoborohydride. The claimed invention, on the other hand, requires the activation of the protein by reaction with hydrazine dihydrochloride at an acidic pH (claim 1). In this reaction, hydrazide groups are introduced into the protein molecule by reaction with the carboxylic groups of amino acids: aspartic acid and glutamic acid by carbodiimide methodology. Schwartz also did not report the possibility of obtaining conjugate vaccine in the lyophilized form (addition of sucrose and lyophilization) as described by Applicants.

B) Applicants argue size exclusion chromatography is not diafiltration. In the purification step of the conjugate, Schwartz describes the use of size exclusion chromatography Superdex 200 column, contrary to the tangential filtration claimed by Applicants. Applicants states the tangential filtration process has advantages over size exclusion chromatography. Applicants state the use of ultrafiltration for both desalting and concentration allows replacing the need for chromatography columns of size exclusion. See, e.g., Larry Schwartz, "Desalting and Buffer Exchange by Dialysis, Gel Filtration, or Diafiltration"
http://www.pall.com/laboratory_42217.asp.

C) The secondary art Ryall et al. cited in each rejection fails to remedy all of the aforementioned deficiencies of Schwartz to teach the claimed invention. Ryall et al. does not teach a neutralizing step, there is a inconsistency in relation to the use of adipic acid dihydrazide

(ADH), since this reagent is used to activate the polysaccharide, which was previously "oxidized" by the action of peroxide hydrogen, by reaction with carbodiimide - EDAC (see column 2, lines 20 to 38, column 3 lines 40 to 45). In Applicants' invention, the reagent ADH is used to neutralize the aldehydes present on the activated polysaccharide which did not react with activated tetanus toxoid during the conjugation reaction (see description and claim 15 of the present invention). Ryall et al. does not teach activation with NaI04. The methodology described by Ryall et al. for conjugation between the polysaccharide and carrier protein, differs from that of Applicants' invention, as the polysaccharide, besides being oxidized/depolymerized, is derivatized with ADH by carbodiimide methodology. Unlike Ryall et al., Applicants oxidize the polysaccharide using NaIO4 when aldehyde groups are generated.

D) Ryall et al. purification procedure differs from that of Applicants' invention, which is carried out by tangential filtration using a filter with a higher molecular weight cutoff (100 kDa is presently preferred) to obtain at least two liters of product called for in claim 1. Applicants' state it must be pointed out that the structural characteristics of the conjugate obtained from the methodology described in the application was the guide for the selection of molecular weight cutoff of the membrane used in the purification step by diafiltration. Applicants state, with respect to the conjugation reaction itself, the patent of Ryall et al. describes that the link between the derivatized polysaccharide and carrier protein derivatized with cystamine, occurs in the presence of EDAC (conjugate by the carbodiimide method), at a temperature of 22 to 23°C for 22 to 24 hours, i.e., the chemical bonding occurs between the amine groups introduced into the polysaccharide and the carboxyl groups present in the protein (see column 8, line 54 to 58 and examples 3 and 9) which occurs in the absence of NaBH3CN.

E) Kuriyama et al. does not teach the use of Hydrazine Dihydrochloride, the description of methodology for obtaining hydrazine with reduced content of organic carbon (impurities) does not correlate with the use of hydrazine dihydrochloride, which is a commercial reagent with a purity degree suitable for obtaining conjugate vaccines.

Examiners Response to Applicants Arguments:

With regard to Points (A) and (C), Although Example 29 does not specifically disclose the aldehyde-activated polysaccharide with the hydrazine activated protein in the presence of sodium cyanoborohydride, Schwartz et al teach specific methods for preparing hydrazino-

containing conjugates comprising aldehyde-activated bacterial polysaccharide with a hydrazine activated protein (see column 22 lines 30-45, column 29 lines 1-40 and column 30 lines 1-20) by utilizing reagents to improve crosslinking for both in vitro and in vivo diagnostic assays as well as in vivo therapies (see abstract). Additionally, Schwartz teach methods for preparing hydrazino-containing conjugates with the addition of a solution of a bifunctional hydrazino modification reagent, wherein the reagent is utilized to form crosslinks between for example protein--protein conjugates or protein-polymer conjugates with sodium cyanoborohydride to form a stable alkylhydrazide bond in a non-nucleophilic buffer within a range of pH 7.0-8.0 (see column 24 lines 43-67). Therefore the method of Schwartz necessarily teach a method for preparing a conjugate, reacting the aldehyde-activated polysaccharide with the hydrazine activated protein in the presence of sodium cyanoborohydride, whereby a conjugate is obtained at a pH of 7 in the presence of the reducing agent sodium cyanoborohydride as evidenced to the contrary. Therefore the limitation has been met.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). The relevance of the teachings Schwartz is aforementioned above. Therefore, applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., Schwartz patent describes the chemical modification of protein by using compounds such as hydrazine and oxiamine, at pH 7-9 (neutral to basic) for a period of 1 to 4 hours are not recited in the rejected claim(s).

Furthermore, Applicants response that Schwartz does not teach obtaining conjugate vaccine in the lyophilized form (addition of sucrose and lyophilization) is unpersuasive because, Ryall et al teach methods for preparing construct comprising the covalent attachment of polysaccharides to protein molecules, wherein said construct is formulated in a suitable carrier with a preservative (i.e. glucose), wherein said construct is lyophilized (see column 13 lines 10-25), which correlates to a method further comprising the step of adding saccharose as a stabilizer yielding a stabilized conjugate vaccine. Therefore one would be motivated at the time the invention was made to made to incorporate the method of adding saccharose as a stabilizer in a

method for preparing a conjugate vaccine using a bacterial polysaccharide (as disclosed by Ryan et al) in the method of Schwartz in order to take advantage of maintaining a stable solution or lyophilization in a composition comprising crosslinking couples in a method for preparing a conjugate vaccine (as disclosed by Schwartz see column 1 lines 45-60).

With regard to Points (B) and (C), in response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Therefore, the relevance of Schwartz teach a method for preparing hydrazino-containing conjugates comprising aldehyde-activated bacterial polysaccharide with a hydrazine activated protein (see column 22 lines 30-45, column 29 lines 1-40 and column 30 lines 1-20) by utilizing reagents to improve crosslinking for both in vitro and in vivo diagnostic assays as well as in vivo therapies (see abstract). Furthermore, in response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., Schwartz teach the use of size exclusion chromatography Superdex 200 column not diafiltration and the advantages over size exclusion chromatography) are not recited in the rejected claim(s). Applicants response that Schwartz does not teach tangential filtration is unpersuasive because Ryall et al teach a method further comprising depolymerized polysaccharide purified by ultrafiltration and a protein molecule which can be purified by ultrafiltration using a FILTRON-type minisette tangential flow filtration unit equipped with a 1,000 molecular weight cutoff (MWCO) Omega modified polyethersulfone screen channel unit cassette (see column 19 lines 40-50). Ryall et al teach a method step of diafiltering a conjugate (see column 19 lines 30-50) which correlates a method, whereby substantially (a relative term) all unreacted compounds and unconjugated polysaccharides are removed yielding a purified conjugate vaccine as evidenced to the contrary because the term "substantially" does not provide a standard for ascertaining the requisite degree in the specification. Furthermore, given that Schwartz and Ryan et al both teach methods for preparing a conjugate vaccines using bacterial polysaccharide and given that Ryan et al teach method steps diafiltering a conjugate, and concentrating the purified conjugate vaccine by tangential flow ultrafiltration are well known in the art with predictable results, it remains obvious to combine the teachings of Schwartz and

Ryan et al, even without an express statement of motivation. KSR forcloses the argument that a specific teaching, suggestion, or motivation is required to support a finding obviousness. See the recent Board Decision Ex parte Smith, --USPQ2d--, slip op. at 20, (Bd. Pat. App. & Interf. June 25, 2007 (citing KSR, 82 USPQ2d at 1396) available at (**http://www.uspto.gov/web/offices/dcom/bpai/prec/fd071925.pdf**).

With regard to Point (D), in response, Moreover, the claims are not limited a method using a filter with a higher molecular weight cutoff (100 kDa is presently preferred) to obtain at least two liters of product carried out by tangential filtration. Moreover, the rejection does not include knowledge gleaned only from Applicant's disclosure. The motivation to combine can be different from Applicants. It is not necessary that the prior art suggest the combination to achieve the same advantage or result discovered by applicant. MPEP 2144(IV). Hence, the fact that applicant has recognized another advantage which would flow naturally from following the suggestion of the prior art cannot be the basis for patentability when the differences would otherwise be obvious. See *Ex parte Obiaya*, 227 USPQ 58, 60 (Bd. Pat. App. & Inter. 1985). Therefore the rejection is maintained.

With regard to Point (E), Kuriyama et al teach hydrazinium monochloride, also known as hydrazine monochloride (as evidenced by Material Safety Data Sheet from Fischer Scientific (see attachment)). Schwartz teaches a method of using hydrazine to activate a protein so it can react with an aldehyde-activated polysaccharide which has carbon constituents. Furthermore given Kuriyama et al teach hydrazine monochloride used in a concentration-distillation process to remove the majority of total organic carbon constituents to recover a purified product in a solution is well known in the art, wherein the use of a hydrazine dichloride in a concentration-distillation process would constitute as an obvious variant and because the obvious variant hydrazine dichloride is well known in the art, leading with predictable results, it remains obvious to combine the teachings of Schwartz and Kuriyama et al to use the obvious variant hydrazine dichloride in the method disclosed in order to take advantage of utilizing the hydrazine bond formed following conjugation in a composition comprising crosslinking couples in a method for preparing a conjugate vaccine (as disclosed by Schwartz column 1 lines 45-60). KSR forcloses the argument that a specific teaching, suggestion, or motivation is required to support a finding obviousness. See the recent Board Decision Ex parte Smith, --USPQ2d--, slip op. at 20, (Bd. Pat.

App. & Interf. June 25, 2007 (citing KSR, 82 USPQ2d at 1396) available at (http://www.uspto.gov/web/offices/dcom/bpai/prec/fd071925.pdf).

As outlined previously, the claims are drawn to a method for preparing a conjugate vaccine in commercial volumes, the method comprising: reacting a polysaccharide with an oxidizing agent, whereby a solution of an aldehyde-activated polysaccharide is obtained; reacting a protein with hydrazine dichloride at an acidic pH, whereby a solution of a hydrazine-activated protein is obtained; purifying said solution of hydrazine-activated protein under conditions standardized to process at least five liters of solution; reacting the aldehyde-activated polysaccharide with the hydrazine-activated protein at a pH of from 5 to 7 in the presence of sodium cyanoborohydride, whereby a conjugate is obtained; neutralizing unreacted aldehyde groups with adipic acid dihydrazide; and purifying the resulting solution under conditions standardized to process a volume of at least two liters, whereby a conjugate vaccine capable of stimulating an immune response is obtained in commercial volumes (claim 1), wherein the oxidizing agent comprises NaIO4 (claim 2), wherein the solution of the aldehyde-activated polysaccharide is buffer exchanged with a HEPES buffer (claim 3), wherein the solution of the aldehyde-activated polysaccharide is buffer exchanged to a pH from 7 to 8 (claim 4), wherein the aldehyde- activated polysaccharide is reacted with the hydrazine-activated protein at a ratio-~-f from 1:1.6 to about 1:5 (claim 8), wherein said purifying the resulting solution comprises the step of diafiltrating the conjugate vaccine, whereby substantially all unreacted compounds and unconjugated polysaccharides are removed, yielding a purified conjugate vaccine (claim 9), , further comprising the step of concentrating the purified conjugate vaccine by tangential flow ultrafiltration, yielding a concentrated purified conjugate vaccine (claim 10), further comprising the step of adding saccharose as a stabilizer to the concentrated purified conjugate vaccine, yielding a stabilized conjugate vaccine (claim 11), further comprising the step of freeze drying the concentrated purified conjugate vaccine, yielding a dried conjugate vaccine (claim 12), wherein the polysaccharide is selected from the group consisting of Meningococcal polysaccharides, Pneumococcus polysaccharides, Hemophilus influenzae type b polysaccharide, Vi polysaccharide of Salmonella typhi, and group B Streptococcus polysaccharides (claim 13), wherein the protein is selected from the group consisting of tetanus toxoid, diptheria toxoid, CRM_{197}, and meningococcal protein (claim 14).

Schwartz teaches a method of immobilizing a biological molecule, comprising: preparing a conjugate bound to a biological molecule, wherein the conjugate is formed from a carrier protein, wherein the biomolecule is a bacterial polysaccharide (see claims and column 3 lines 55-65). Schwartz teaches incorporating a carbonyl group on a biomolecule to produce dialdehydes by periodate-mediated oxidation (see column 22 lines 30-45). Schwartz further teach a bacterial polysaccharide, oxidized with sodium periodate to form dialdehyde moieties (see Example 29 column 34, column 29 lines 1-40 and column 30 lines 1-20), which correlates to a method for preparing a conjugate, whereby a solution of an aldehyde-activated polysaccharide is obtained. Schwartz et al teach specific methods for preparing hydrazino-containing conjugates comprising aldehyde-activated bacterial polysaccharide with a hydrazine activated protein (see column 22 lines 30-45, column 29 lines 1-40 and column 30 lines 1-20) by utilizing reagents to improve crosslinking for both in vitro and in vivo diagnostic assays as well as in vivo therapies (see abstract). Additionally, Schwartz teach methods for preparing hydrazino-containing conjugates with the addition of a solution of a bifunctional hydrazino modification reagent, wherein the reagent is utilized to form crosslinks between for example protein--protein conjugates or protein-polymer conjugates with sodium cyanoborohydride to form a stable alkylhydrazide bond in a non-nucleophilic buffer within a range of pH 7.0-8.0 (see column 24 lines 43-67). Therefore the method of Schwartz necessarily teach a method for preparing a conjugate, reacting the aldehyde-activated polysaccharide with the hydrazine activated protein in the presence of sodium cyanoborohydride, whereby a conjugate is obtained at a pH of 7 in the presence of sodium cyanoborohydride and wherein the solution of the aldehyde-activated polysaccharide is a buffer exchanged with a HEPES buffer as evidenced by Behr et al (see pg. 546 first paragraph) as evidenced to the contrary. Schwartz teaches modifying protein carriers such as tetanus toxoid or diphtheria toxoid (column 20 lines 1-5), binding a protein with a hydrazine in a buffer solution at a pH of 4.7 (see Figure 1, 4. Bifunctional Carbonyl Reagents Section, Example 11), which correlates to method comprising the binding of a protein with hydrazine at an acidic pH, whereby a solution of a hydrazine-activated protein is obtained. Schwartz teaches a method of forming crosslinks between protein-polymer conjugates (column 24 lines 50-65 and (column 25 lines 1-10) in the presence of sodium cyanoborohydride (see column 24 lines 60-67)) between a range of 4.7 and 7.4 (see column 25 lines 30-45). Schwartz teaches an aldehyde-activated polysaccharide

with concentration of 5 mg/ml (see Example 29) and hydrazine-activated protein polysaccharide with concentration 5mg/ml (Example 11) at a ratio of 1:1. Schwartz teaches a method of preparing conjugates for in vivo uses as vaccines, thus the method of Schwartz would necessarily teach a conjugate vaccines in commercial volumes, wherein a conjugate vaccine is capable of stimulating an immune response obtained in commercial volumes as evidenced to the contrary.

Schwartz does not teach a method for preparing a conjugate comprising method steps, reacting a protein specifically with hydrazine dichloride in a solution, purifying the solution of hydrazine-activated protein under conditions standardized to process at least five liters of solution, neutralizing unreacted aldehyde groups with adipic acid dihydrazide; and purifying the resulting solution under conditions standardized to process a volume of at least two liters, wherein said purifying the resulting solution comprises further comprising the step of diafiltrating the conjugate vaccine, whereby substantially all unreacted compounds and unconjugated polysaccharides are removed, yielding a purified conjugate vaccine, further comprising the step of concentrating the purified conjugate vaccine by tangential flow ultrafiltration, yielding a concentrated purified conjugate vaccine, further comprising the step of adding saccharose as a stabilizer to the concentrated purified conjugate vaccine, yielding a stabilized conjugate vaccine, further comprising the step of freeze drying the concentrated purified conjugate vaccine, yielding a dried conjugate vaccine, wherein the polysaccharide is selected from the group consisting of Meningococcal polysaccharides, Pneumococcus polysaccharides, *Hemophilus influenzae type b* polysaccharide, Vi polysaccharide of *Salmonnella typhi*, and *Group B Streptococcus* polysaccharides.

Ryall et al teach a method for preparing a construct comprising a bacterial polysaccharide comprising Streptococcus pneumoniae covalently attached to a protein molecule (see claims and column 15 lines 20-25), comprising mixing unreduced depolymerized polysaccharide chains with adipic dihydrazide (see column 7 lines 30-35), which correlates to neutralizing unreacted aldehyde groups with adipic acid dihydrazide and purifying the resulting solution under conditions standardized to process a volume of at least two liters. Ryall et al teach a method step of diafiltering a conjugate (see column 19 lines 30-50), whereby substantially all unreacted compounds and unconjugated polysaccharides are removed yielding a purified conjugate vaccine. Ryall et al teach a method further comprising depolymerized polysaccharide purified by

ultrafiltration and a protein molecule which can be purified by ultrafiltration using a FILTRON-type minisette tangential flow filtration unit equipped with a 1,000 molecular weight cutoff (MWCO) Omega modified polyethersulfone screen channel unit cassette (see column 19 lines 40-50), which correlate to a method of yielding a concentrated purified conjugate vaccine, a method comprising purifying the resulting solution under conditions standardized to process at least five liters of solution. Ryall et al teach compositions comprising the construct may be in admixture with a suitable carrier glucose or the like, which correlate to a method further comprising the step of adding saccharose as a stabilizer to the concentrated purified vaccine yielding a stabilized conjugate vaccine.

Kuriyama et al teach hydrazinium monochloride, also known as hydrazine monochloride (as evidenced by Material Safety Data Sheet from Fischer Scientific (see attachment). Moreover Kuriyama et al teach hydrazinium monochloride are preferably usable in a concentration-distillation process comprising distilling an aqueous solution of a product in the presence of hydrazinium monochloride to concentrate the aqueous solution of a product by distilling water and the majority of the total organic carbon constituents off and further separating the resultant concentrate as a bottom product, to further comprise distilling the resultant concentrate to recover a purified aqueous solution as a top product and separating an aqueous solution of the above salt as a bottom product in a solution (see column 3 lines 1-35 column 6 lines 1-8).

Schwartz and Ryan et al both teach methods for preparing a conjugate vaccines using bacterial polysaccharide. Furthermore, Ryan et al teach *Streptococcus pneumoniae* is used as a bacterial polysaccharide in a method for preparing a conjugate vaccine. Moreover, Ryan et al teach method steps comprising neutralizing unreacted aldehyde groups with adipic acid dihydrazide and purifying the resulting solution, diafiltering a conjugate, and concentrating the purified conjugate vaccine by tangential flow ultrafiltration, further comprising the step of adding saccharose as a stabilizer in a method for preparing a conjugate vaccine. Therefore the use of *Streptococcus pneumoniae* constitutes an obvious variant of the method disclosed by Schwartz. Moreover since the use of *Streptococcus pneumoniae* is known in the art with predictable results it is obvious to use it in the method of Schwartz. Also the use of the method steps of Ryan et al are well known in the art with predictable results, thus it remains obvious to combine the teachings of Schwartz and Ryan et al, even without an express statement of

motivation. KSR forcloses the argument that a specific teaching, suggestion, or motivation is required to support a finding obviousness. See the recent Board Decision Ex parte Smith, -- USPQ2d--, slip op. at 20, (Bd. Pat. App. & Interf. June 25, 2007 (citing KSR, 82 USPQ2d at 1396) available at (**http://www.uspto.gov/web/offices/dcom/bpai/prec/fd071925.pdf**).

Schwartz teaches a method of using hydrazine to activate a protein so it can react with an aldehyde-activated polysaccharide which has carbon constituents. Kuriyama et al teach hydrazinium monochloride, also known as hydrazine monochloride (as evidenced by Material Safety Data Sheet from Fischer Scientific (see attachment). Furthermore given Kuriyama et al teach hydrazine monochloride used in a concentration-distillation process to remove the majority of total organic carbon constituents to recover a purified product in a solution is well known in the art, wherein the use of a hydrazine dichloride in a concentration-distillation process would constitute as an obvious variant and because the obvious variant hydrazine dichloride is well known in the art, leading with predictable results, it remains obvious to combine the teachings of Schwartz and Kuriyama et al to use the obvious variant hydrazine dichloride in the method disclosed in order to take advantage of utilizing the hydrazine bond formed following conjugation in a composition comprising crosslinking couples in a method for preparing a conjugate vaccine (as disclosed by Schwartz column 1 lines 45-60). KSR forcloses the argument that a specific teaching, suggestion, or motivation is required to support a finding obviousness. See the recent Board Decision Ex parte Smith, --USPQ2d--, slip op. at 20, (Bd. Pat. App. & Interf. June 25, 2007 (citing KSR, 82 USPQ2d at 1396) available at (**http://www.uspto.gov/web/offices/dcom/bpai/prec/fd071925.pdf**).

5. The rejection of claims 1-20 under 35 U.S.C. 103(a) as being unpatentable over (Schwartz US Patent No. 6,800,728 Date October 5, 2004 US Filing Date March 22, 2001), (Ryall et al US Patent No. 5,965,714 Date October 12, 1999), (Kuriyama et al US Patent No. 4,963,232 Date October 16, 1990), and (Donovan et al US Patent No. 5,480,643 Date January 2, 1996, Powell US Patent No. 5,066408 Date November 19, 1991), as evidenced by (Behr et al 2003 Tetrahedron 59 pgs. 543-553) is maintained for the reasons set forth in the previous office action.

Applicants arguments filed in response to the 35 U.S.C. 103(a), April 7, 2010 is carefully considered, but not found to be persuasive for the reasons below.

Applicant argues:

A) Schwartz does not disclose all claimed features. Although Schwartz describes the conjugation between oxidized polysaccharide and NaIO4 and modified protein with hydrazine (see example 29), the reaction does not occur by the mechanism of reductive amination, i.e., the connection between the protein modified by hydrazine and polysaccharide activated by NaIO4 occurs in the absence of reducing agent. Thus, Schwartz fails to disclose or suggest the claimed feature of reacting the aldehyde-activate polysaccharide with the hydrazine-activated protein at a pH of from 5 to 7 in the presence of the reducing agent sodium cyanoborohydride. The claimed invention, on the other hand, requires the activation of the protein by reaction with hydrazine dihydrochloride at an acidic pH (claim 1). In this reaction, hydrazide groups are introduced into the protein molecule by reaction with the carboxylic groups of amino acids: aspartic acid and glutamic acid by carbodiimide methodology. Schwartz also did not report the possibility of obtaining conjugate vaccine in the lyophilized form (addition of sucrose and lyophilization) as described by Applicants.

B) Applicants argue size exclusion chromatography is not diafiltration. In the purification step of the conjugate, Schwartz describes the use of size exclusion chromatography Superdex 200 column, contrary to the tangential filtration claimed by Applicants. Applicants states the tangential filtration process has advantages over size exclusion chromatography. Applicants state the use of ultrafiltration for both desalting and concentration allows replacing the need for chromatography columns of size exclusion. See, e.g., Larry Schwartz, "Desalting and Buffer Exchange by Dialysis, Gel Filtration, or Diafiltration" http://www.pall.com/laboratory_42217.asp.

C) The secondary art Ryall et al. cited in each rejection fails to remedy all of the aforementioned deficiencies of Schwartz to teach the claimed invention. Ryall et al. does not teach a neutralizing step, there is a inconsistency in relation to the use of adipic acid dihydrazide (ADH), since this reagent is used to activate the polysaccharide, which was previously "oxidized" by the action of peroxide hydrogen, by reaction with carbodiimide - EDAC (see column 2, lines 20 to 38, column 3 lines 40 to 45). In Applicants' invention, the reagent ADH is

used to neutralize the aldehydes present on the activated polysaccharide which did not react with activated tetanus toxoid during the conjugation reaction (see description and claim 15 of the present invention). Ryall et al. does not teach activation with NaI04. The methodology described by Ryall et al. for conjugation between the polysaccharide and carrier protein, differs from that of Applicants' invention, as the polysaccharide, besides being oxidized/depolymerized, is derivatized with ADH by carbodiimide methodology. Unlike Ryall et al., Applicants oxidize the polysaccharide using NaIO4 when aldehyde groups are generated.

D) Ryall et al. purification procedure differs from that of Applicants' invention, which is carried out by tangential filtration using a filter with a higher molecular weight cutoff (100 kDa is presently preferred) to obtain at least two liters of product called for in claim 1. Applicants' state it must be pointed out that the structural characteristics of the conjugate obtained from the methodology described in the application was the guide for the selection of molecular weight cutoff of the membrane used in the purification step by diafiltration. Applicants state, with respect to the conjugation reaction itself, the patent of Ryall et al. describes that the link between the derivatized polysaccharide and carrier protein derivatized with cystamine, occurs in the presence of EDAC (conjugate by the carbodiimide method), at a temperature of 22 to 23°C for 22 to 24 hours, i.e., the chemical bonding occurs between the amine groups introduced into the polysaccharide and the carboxyl groups present in the protein (see column 8, line 54 to 58 and examples 3 and 9) which occurs in the absence of NaBH3CN.

E) Kuriyama et al. does not teach the use of Hydrazine Dihydrochloride, the description of methodology for obtaining hydrazine with reduced content of organic carbon (impurities) does not correlate with the use of hydrazine dihydrochloride, which is a commercial reagent with a purity degree suitable for obtaining conjugate vaccines.

F) Regardless of whether or not the Office is correct that Donovan et al. and Powell et al. teach the use of sodium carbonate as a buffer, these references still fail to remedy the aforementioned deficiencies of the other references to teach all the limitations of the claimed invention.

Examiners Response to Applicants Arguments:

With regard to Points (A), (C), and (F), Although Example 29 does not specifically disclose the aldehyde-activated polysaccharide with the hydrazine activated protein in the

presence of sodium cyanoborohydride, Schwartz et al teach specific methods for preparing hydrazino-containing conjugates comprising aldehyde-activated bacterial polysaccharide with a hydrazine activated protein (see column 22 lines 30-45, column 29 lines 1-40 and column 30 lines 1-20) by utilizing reagents to improve crosslinking for both in vitro and in vivo diagnostic assays as well as in vivo therapies (see abstract). Additionally, Schwartz teach methods for preparing hydrazino-containing conjugates with the addition of a solution of a bifunctional hydrazino modification reagent, wherein the reagent is utilized to form crosslinks between for example protein--protein conjugates or protein-polymer conjugates with sodium cyanoborohydride to form a stable alkylhydrazide bond in a non-nucleophilic buffer within a range of pH 7.0-8.0 (see column 24 lines 43-67). Therefore the method of Schwartz necessarily teach a method for preparing a conjugate, reacting the aldehyde-activated polysaccharide with the hydrazine activated protein in the presence of sodium cyanoborohydride, whereby a conjugate is obtained at a pH of 7 in the presence of the reducing agent sodium cyanoborohydride as evidenced to the contrary. Therefore the limitation has been met.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). The relevance of the teachings Schwartz is aforementioned above. Therefore, applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., Schwartz patent describes the chemical modification of protein by using compounds such as hydrazine and oxiamine, at pH 7-9 (neutral to basic) for a period of 1 to 4 hours are not recited in the rejected claim(s).

Furthermore, Applicants response that Schwartz does not teach obtaining conjugate vaccine in the lyophilized form (addition of sucrose and lyophilization) is unpersuasive because, Ryall et al teach methods for preparing construct comprising the covalent attachment of polysaccharides to protein molecules, wherein said construct is formulated in a suitable carrier with a preservative (i.e. glucose), wherein said construct is lyophilized (see column 13 lines 10-25), which correlates to a method further comprising the step of adding saccharose as a stabilizer yielding a stabilized conjugate vaccine. Therefore one would be motivated at the time the

invention was made to made to incorporate the method of adding saccharose as a stabilizer in a method for preparing a conjugate vaccine using a bacterial polysaccharide (as disclosed by Ryan et al) in the method of Schwartz in order to take advantage of maintaining a stable solution or lyophilization in a composition comprising crosslinking couples in a method for preparing a conjugate vaccine (as disclosed by Schwartz see column 1 lines 45-60).

With regard to Points (B), (C), and (F), in response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Therefore, the relevance of Schwartz teach a method for preparing hydrazino-containing conjugates comprising aldehyde-activated bacterial polysaccharide with a hydrazine activated protein (see column 22 lines 30-45, column 29 lines 1-40 and column 30 lines 1-20) by utilizing reagents to improve crosslinking for both in vitro and in vivo diagnostic assays as well as in vivo therapies (see abstract). Furthermore, in response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., Schwartz teach the use of size exclusion chromatography Superdex 200 column not diafiltration and the advantages over size exclusion chromatography) are not recited in the rejected claim(s). Applicants response that Schwartz does not teach tangential filtration is unpersuasive because Ryall et al teach a method further comprising depolymerized polysaccharide purified by ultrafiltration and a protein molecule which can be purified by ultrafiltration using a FILTRON-type minisette tangential flow filtration unit equipped with a 1,000 molecular weight cutoff (MWCO) Omega modified polyethersulfone screen channel unit cassette (see column 19 lines 40-50). Ryall et al teach a method step of diafiltering a conjugate (see column 19 lines 30-50) which correlates a method, whereby substantially all unreacted compounds and unconjugated polysaccharides are removed yielding a purified conjugate vaccine as evidenced to the contrary because the term "substantially" does not provide a standard for ascertaining the requisite degree in the specification. Furthermore, given that Schwartz and Ryan et al both teach methods for preparing a conjugate vaccines using bacterial polysaccharide and given that Ryan et al teach method steps diafiltering a conjugate, and concentrating the purified conjugate vaccine by tangential flow ultrafiltration are well known in the art with predictable

results, it remains obvious to combine the teachings of Schwartz and Ryan et al, even without an express statement of motivation. KSR forcloses the argument that a specific teaching, suggestion, or motivation is required to support a finding obviousness. See the recent Board Decision Ex parte Smith, --USPQ2d--, slip op. at 20, (Bd. Pat. App. & Interf. June 25, 2007 (citing KSR, 82 USPQ2d at 1396) available at (**http://www.uspto.gov/web/offices/dcom/bpai/prec/fd071925.pdf**).

With regard to Point (D), in response, Moreover, the claims are not limited a method using a filter with a higher molecular weight cutoff (100 kDa is presently preferred) to obtain at least two liters of product carried out by tangential filtration. Moreover, the rejection does not include knowledge gleaned only from Applicant's disclosure. The motivation to combine can be different from Applicants. It is not necessary that the prior art suggest the combination to achieve the same advantage or result discovered by applicant. MPEP 2144(IV). Hence, the fact that applicant has recognized another advantage which would flow naturally from following the suggestion of the prior art cannot be the basis for patentability when the differences would otherwise be obvious. See *Ex parte Obiaya*, 227 USPQ 58, 60 (Bd. Pat. App. & Inter. 1985). Therefore the rejection is maintained.

With regard to Point (E), Kuriyama et al teach hydrazinium monochloride, also known as hydrazine monochloride (as evidenced by Material Safety Data Sheet from Fischer Scientific (see attachment)). Schwartz teaches a method of using hydrazine to activate a protein so it can react with an aldehyde-activated polysaccharide which has carbon constituents. Furthermore given Kuriyama et al teach hydrazine monochloride used in a concentration-distillation process to remove the majority of total organic carbon constituents to recover a purified product in a solution is well known in the art, wherein the use of a hydrazine dichloride in a concentration-distillation process would constitute as an obvious variant and because the obvious variant hydrazine dichloride is well known in the art, leading with predictable results, it remains obvious to combine the teachings of Schwartz and Kuriyama et al to use the obvious variant hydrazine dichloride in the method disclosed in order to take advantage of utilizing the hydrazine bond formed following conjugation in a composition comprising crosslinking couples in a method for preparing a conjugate vaccine (as disclosed by Schwartz column 1 lines 45-60). KSR forcloses the argument that a specific teaching, suggestion, or motivation is required to support a finding

obviousness. See the recent Board Decision Ex parte Smith, --USPQ2d--, slip op. at 20, (Bd. Pat. App. & Interf. June 25, 2007 (citing KSR, 82 USPQ2d at 1396) available at (**http://www.uspto.gov/web/offices/dcom/bpai/prec/fd071925.pdf**).

As outlined previously, the claims are drawn to a method for preparing a conjugate vaccine in commercial volumes, the method comprising: reacting a polysaccharide with an oxidizing agent, whereby a solution of an aldehyde-activated polysaccharide is obtained; reacting a protein with hydrazine dichloride at an acidic pH, whereby a solution of a hydrazine-activated protein is obtained; purifying said solution of hydrazine-activated protein under conditions standardized to process at least five liters of solution; reacting the aldehyde-activated polysaccharide with the hydrazine-activated protein at a pH of from 5 to 7 in the presence of sodium cyanoborohydride, whereby a conjugate is obtained; neutralizing unreacted aldehyde groups with adipic acid dihydrazide; and purifying the resulting solution under conditions standardized to process a volume of at least two liters, whereby a conjugate vaccine capable of stimulating an immune response is obtained in commercial volumes (claim 1), wherein the oxidizing agent comprises NalO4 (claim 2), wherein the solution of the aldehyde-activated polysaccharide is buffer exchanged with a HEPES buffer (claim 3), wherein the solution of the aldehyde-activated polysaccharide is buffer exchanged to a pH from 7 to 8 (claim 4), wherein the solution of the hydrazine- activated protein is buffer exchanged with a NazCO3 buffer (claim 5), wherein the solution of the I hydrazine-activated protein is buffer exchanged to a pH of from 10.0 to 11.0. (claim 6), wherein a pH of the solution of the hydrazine-activated protein is raised from 7.0 to 11 before the solution of the hydrazine-activated protein is buffer exchanged to a pH of from 10.0 to 11.0 (claim 7), wherein the aldehyde- activated polysaccharide is reacted with the hydrazine-activated protein at a ratio from 1:1.6 to about 1:5 (claim 8), wherein said purifying the resulting solution comprises the step of diafiltrating the conjugate vaccine, whereby substantially all unreacted compounds and unconjugated polysaccharides are removed, yielding a purified conjugate vaccine (claim 9), , further comprising the step of concentrating the purified conjugate vaccine by tangential flow ultrafiltration, yielding a concentrated purified conjugate vaccine (claim 10), further comprising the step of adding saccharose as a stabilizer to the concentrated purified conjugate vaccine, yielding a stabilized conjugate vaccine (claim 11), further comprising the step of freeze drying the concentrated purified conjugate vaccine, yielding

a dried conjugate vaccine (claim 12), wherein the polysaccharide is selected from the group consisting of Meningococcal polysaccharides, Pneumococcus polysaccharides, Hemophilus influenzae type b polysaccharide, Vi polysaccharide of Salmonnella typhi, and group B Streptococcus polysaccharides (claim 13), wherein the protein is selected from the group consisting of tetanus toxoid, diptheria toxoid, CRM_{197}, and meningococcal protein (claim 14); a method for preparing a conjugate vaccine in commercial volumes, the method comprising: reacting a polysaccharide with an oxidizing agent, whereby a solution of an aldehyde-activated polysaccharide is obtained; buffer exchanging the solution of the aldehyde-activated polysaccharide to a pH of from about 7 to 8; reacting a protein with hydrazine dichloride at an acidic pH, whereby a solution of a hydrazine-activated protein is obtained; raising a pH of the solution of the hydrazine-activated protein from 7.0 to 11 and thereafter buffer exchanging the solution of the hydrazine-activated protein to a pH of from 10.0 to 11.0; purifying said solution of hydrazine-activated protein under conditions standardized to process at least five liters of solution reacting the aldehyde-activated polysaccharide with the hydrazine-activated protein at a pH of from 5 to 7 in the presence of sodium cyanoborohydride, whereby a conjugate is obtained; neutralizing unreacted aldehyde groups with adipic acid dihydrazide; and purifying the resulting solution under conditions standardized to process a volume of at least two liters, whereby a conjugate vaccine capable of stimulating an immune response is obtained in commercial volumes (claim 15), wherein the aldehyde-activated polysaccharide is reacted with the hydrazine-activated protein at a ratio from about 1:1.6 to about 1:5 (claim 16), wherein said purifying the resulting solution comprises the step of diafiltrating the conjugate vaccine, whereby substantially all unreacted compounds and unconjugated polysaccharides are removed, yielding a purified conjugate vaccine (claim 17), further comprising the step of concentrating the purified conjugate vaccine by tangential flow ultrafiltration, yielding a concentrated purified conjugate vaccine (claim 18), further comprising the step of adding saccharose as a stabilizer to the concentrated purified conjugate vaccine, yielding a stabilized conjugate vaccine (claim 19), wherein the polysaccharide is selected from the group consisting of Meningococcal polysaccharides, Pneumococcus polysaccharides, Hemophilus influenzae type b polysaccharide, Vi polysaccharide of Salmonnella typhi, and group B Streptococcus polysaccharides, and

wherein the protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, CRM197, and meningococcal protein (claim 20).

Schwartz teaches a method of immobilizing a biological molecule, comprising: preparing a conjugate bound to a biological molecule, wherein the conjugate is formed from a carrier protein, wherein the biomolecule is a bacterial polysaccharide (see claims and column 3 lines 55-65). Schwartz teaches incorporating a carbonyl group on a biomolecule to produce dialdehydes by periodate-mediated oxidation (see column 22 lines 30-45). Schwartz further teach a bacterial polysaccharide, oxidized with sodium periodate to form dialdehyde moieties (see Example 29 column 34, column 29 lines 1-40 and column 30 lines 1-20), which correlates to a method for preparing a conjugate, whereby a solution of an aldehyde-activated polysaccharide is obtained. Schwartz et al teach specific methods for preparing hydrazino-containing conjugates comprising aldehyde-activated bacterial polysaccharide with a hydrazine activated protein (see column 22 lines 30-45, column 29 lines 1-40 and column 30 lines 1-20) by utilizing reagents to improve crosslinking for both in vitro and in vivo diagnostic assays as well as in vivo therapies (see abstract). Additionally, Schwartz teach methods for preparing hydrazino-containing conjugates with the addition of a solution of a bifunctional hydrazino modification reagent, wherein the reagent is utilized to form crosslinks between for example protein--protein conjugates or protein-polymer conjugates with sodium cyanoborohydride to form a stable alkylhydrazide bond in a non-nucleophilic buffer within a range of pH 7.0-8.0 (see column 24 lines 43-67). Therefore the method of Schwartz necessarily teach a method for preparing a conjugate, reacting the aldehyde-activated polysaccharide with the hydrazine activated protein in the presence of sodium cyanoborohydride, whereby a conjugate is obtained at a pH of 7 in the presence of sodium cyanoborohydride and wherein the solution of the aldehyde-activated polysaccharide is a buffer exchanged with a HEPES buffer as evidenced by Behr et al (see pg. 546 first paragraph) as evidenced to the contrary. Schwartz teaches modifying protein carriers such as tetanus toxoid or diphtheria toxoid (column 20 lines 1-5), binding a protein with a hydrazine in a buffer solution at a pH of 4.7 (see Figure 1, 4. Bifunctional Carbonyl Reagents Section, Example 11), which correlates to method comprising the binding of a protein with hydrazine at an acidic pH, whereby a solution of a hydrazine-activated protein is obtained. Schwartz teaches a method of forming crosslinks between protein-polymer conjugates (column 24 lines 50-65 and (column 25 lines 1-

10) in the presence of sodium cyanoborohydride (see column 24 lines 60-67)) between a range of 4.7 and 7.4 (see column 25 lines 30-45). Schwartz teaches an aldehyde-activated polysaccharide with concentration of 5 mg/ml (see Example 29) and hydrazine-activated protein polysaccharide with concentration 5mg/ml (Example 11) at a ratio of 1:1. Schwartz teaches a method of preparing conjugates for in vivo uses as vaccines, thus the method of Schwartz would necessarily teach a conjugate vaccines in commercial volumes, wherein a conjugate vaccine is capable of stimulating an immune response obtained in commercial volumes as evidenced to the contrary.

Schwartz does not teach a method for preparing a conjugate comprising method steps, reacting a protein specifically with hydrazine dichloride in a solution, purifying the solution of hydrazine-activated protein under conditions standardized to process at least five liters of solution, neutralizing unreacted aldehyde groups with adipic acid dihydrazide; and purifying the resulting solution under conditions standardized to process a volume of at least two liters, wherein the solution of the hydrazine-activated protein is buffer exchanged with a Na2CO3 buffer, wherein the solution of the hydrazine-activated protein is buffer exchanged to a pH of from about 10.0 to about 11.0, wherein a pH of the solution of the hydrazine-activated protein is raised to from about 7.0 to about 11 before the solution of the hydrazine-activated protein is buffer exchanged to a pH of from about 10.0 to about 11.0, wherein said purifying the resulting solution comprises further comprising the step of diafiltrating the conjugate vaccine, whereby substantially all unreacted compounds and unconjugated polysaccharides are removed, yielding a purified conjugate vaccine, further comprising the step of concentrating the purified conjugate vaccine by tangential flow ultrafiltration, yielding a concentrated purified conjugate vaccine, further comprising the step of adding saccharose as a stabilizer to the concentrated purified conjugate vaccine, yielding a stabilized conjugate vaccine, further comprising the step of freeze drying the concentrated purified conjugate vaccine, yielding a dried conjugate vaccine, wherein the polysaccharide is selected from the group consisting of Meningococcal polysaccharides, Pneumococcus polysaccharides, *Hemophilus influenzae type b* polysaccharide, Vi polysaccharide of *Salmonnella typhi*, and *Group B Streptococcus* polysaccharides.

Ryall et al teach a method for preparing a construct comprising a bacterial polysaccharide comprising Streptococcus pneumoniae covalently attached to a protein molecule (see claims and column 15 lines 20-25), comprising mixing unreduced depolymerized polysaccharide chains

with adipic dihydrazide (see column 7 lines 30-35), which correlates to neutralizing unreacted aldehyde groups with adipic acid dihydrazide and purifying the resulting solution under conditions standardized to process a volume of at least two liters. Ryall et al teach a method step of diafiltering a conjugate (see column 19 lines 30-50), whereby substantially all unreacted compounds and unconjugated polysaccharides are removed yielding a purified conjugate vaccine. Ryall et al teach a method further comprising depolymerized polysaccharide purified by ultrafiltration and a protein molecule which can be purified by ultrafiltration using a FILTRON-type minisette tangential flow filtration unit equipped with a 1,000 molecular weight cutoff (MWCO) Omega modified polyethersulfone screen channel unit cassette (see column 19 lines 40-50), which correlate to a method of yielding a concentrated purified conjugate vaccine, a method comprising purifying the resulting solution under conditions standardized to process at least five liters of solution. Ryall et al teach compositions comprising the construct may be in admixture with a suitable carrier glucose or the like, which correlate to a method further comprising the step of adding saccharose as a stabilizer to the concentrated purified vaccine yielding a stabilized conjugate vaccine.

Kuriyama et al teach hydrazinium monochloride, also known as hydrazine monochloride (as evidenced by Material Safety Data Sheet from Fischer Scientific (see attachment). Moreover Kuriyama et al teach hydrazinium monochloride are preferably usable in a concentration-distillation process comprising distilling an aqueous solution of a product in the presence of hydrazinium monochloride to concentrate the aqueous solution of a product by distilling water and the majority of the total organic carbon constituents off and further separating the resultant concentrate as a bottom product, to further comprise distilling the resultant concentrate to recover a purified aqueous solution as a top product and separating an aqueous solution of the above salt as a bottom product in a solution (see column 3 lines 1-35 column 6 lines 1-8).

Donovan et al teach a buffering agent to maintain a pH range for optimum activity when a dialdehyde composition is employed in an active form (see column 3 lines 20-28). Donovan et al teach a buffering agent such as sodium carbonate is preferable to add to maintain the pH at an optimum alkaline level for dialdehyde activity (see column 7 lines 59-67 column 8 lines 1-5).

Powell teach it is necessary to add an alkalyzer such as sodium carbonate (soda ash) to raise the pH to prevent the pH from dropping below 7.2 (see column 1 lines 65-69 and column 2 lines 1-10).

As to the limitations of dependent claims 6-7, a method wherein the solution of the hydrazine-activated protein is buffer exchanged to a pH of from about 10 to about 11.0 (claim 6), wherein the hydrazine-activated protein is raised from about 7.0 to about 11 before the solution of the hydrazine-activated protein is buffer exchanged to a pH of from about 10.0 to about 11.0 (claim 7). The references of Donovan et al and Powell et al do not specifically teach a buffer exchanged to a pH of from about 10 to about 11.0, wherein a pH solution is raised from about 7.0 to about 11 before the solution of the hydrazine-activated protein is buffer exchanged to a pH of from about 10.0 to about 11.0 as claimed by the Applicants. The pH of a specific value is clearly a result effective parameter that a person of ordinary skill in the art would routinely optimize. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." In re Aller, 220 F.2d 454,456, 105 USPQ 233, 235 (CCPA 1955). Thus, optimization of general conditions is a routine practice that would be obvious for a person of ordinary skill in the art to employ. It would have been customary for an artisan of ordinary skill to determine the optimal pH in order to best achieve the desired results. Thus, absent some demonstration of unexpected results from the claimed parameters, this optimization of pH would have been obvious at the time of applicant's invention.

Schwartz and Ryan et al both teach methods for preparing a conjugate vaccines using bacterial polysaccharide. Furthermore, Ryan et al teach *Streptococcus pneumoniae* is used as a bacterial polysaccharide in a method for preparing a conjugate vaccine. Moreover, Ryan et al teach method steps comprising neutralizing unreacted aldehyde groups with adipic acid dihydrazide and purifying the resulting solution, diafiltering a conjugate, and concentrating the purified conjugate vaccine by tangential flow ultrafiltration, further comprising the step of adding saccharose as a stabilizer in a method for preparing a conjugate vaccine. Therefore the use of *Streptococcus pneumoniae* constitutes an obvious variant of the method disclosed by Schwartz. Moreover since the use of *Streptococcus pneumoniae* is known in the art with predictable results it is obvious to use it in the method of Schwartz. Also the use of the method

steps of Ryan et al are well known in the art with predictable results, thus it remains obvious to combine the teachings of Schwartz and Ryan et al, even without an express statement of motivation. KSR forcloses the argument that a specific teaching, suggestion, or motivation is required to support a finding obviousness. See the recent Board Decision Ex parte Smith, --USPQ2d--, slip op. at 20, (Bd. Pat. App. & Interf. June 25, 2007 (citing KSR, 82 USPQ2d at 1396) available at (**http://www.uspto.gov/web/offices/dcom/bpai/prec/fd071925.pdf**).

Schwartz teaches a method of using hydrazine to activate a protein so it can react with an aldehyde-activated polysaccharide which has carbon constituents. Kuriyama et al teach hydrazinium monochloride, also known as hydrazine monochloride (as evidenced by Material Safety Data Sheet from Fischer Scientific (see attachment). Furthermore given Kuriyama et al teach hydrazine monochloride used in a concentration-distillation process to remove the majority of total organic carbon constituents to recover a purified product in a solution is well known in the art, wherein the use of a hydrazine dichloride in a concentration-distillation process would constitute as an obvious variant and because the obvious variant hydrazine dichloride is well known in the art, leading with predictable results, it remains obvious to combine the teachings of Schwartz and Kuriyama et al to use the obvious variant hydrazine dichloride in the method disclosed in order to take advantage of utilizing the hydrazine bond formed following conjugation in a composition comprising crosslinking couples in a method for preparing a conjugate vaccine (as disclosed by Schwartz column 1 lines 45-60). KSR forcloses the argument that a specific teaching, suggestion, or motivation is required to support a finding obviousness. See the recent Board Decision Ex parte Smith, --USPQ2d--, slip op. at 20, (Bd. Pat. App. & Interf. June 25, 2007 (citing KSR, 82 USPQ2d at 1396) available at (**http://www.uspto.gov/web/offices/dcom/bpai/prec/fd071925.pdf**).

It would have been prima facie obvious at the time the invention was made to use a buffer exchange with a sodium carbonate as taught by Donovan et al in the method as taught by Schwartz in order to take advantage of maintaining the pH of the hydrazine activated protein at an optimum alkaline level for dialdehyde activity.

One would have had reasonable expectation of success because sodium carbonate is used as a buffering agent (disclosed by Donovan et al) and has been shown to maintain a pH at an alkaline level, as being well known in the art.

Conclusion

6. No claims are allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a). A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Nina A. Archie whose telephone number is 571-272-9938. The examiner can normally be reached on Monday-Friday 8:30-5:00p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner supervisor, Robert Mondesi can be reached on 571-272-0956. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system.

Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see **http://pair-direct.uspto.gov**.

Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Nina A Archie

Examiner

GAU 1645

REM 3B31

/Robert A. Zeman/
for Nina Archie, Examiner of Art Unit 1645